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(54) Title: PURIFIED PROTEINS, RECOMBINANT DNA SEQUENCES AND PROCESSES FOR CONTROLLING THE RIPENING OF COFFEE PLANTS

(57) Abstract

Purified proteins, DNA sequences that code on expression therefore and recombinant DNA molecules, including hosts transformed therewith for transforming coffee plants to suppress the expression of enzymes necessary for ethylene synthesis. The DNA sequences and recombinant DNA molecules are characterized in that they code on expression for the enzymes ACC synthase or ACC oxidase that are elements of the pathway for ethylene biosynthesis in coffee plants. Coffee plants are transformed with vectors containing ACC synthase and/or with ACC oxidate DNA sequences that code on expression for the respective mRNA that is antisense to the mRNA for ACC synthase and/or ACC oxidase. The resulting antisense mRNA binds to XMI mRNA, thereby inactivating the mRNA encoding one or more enzymes in the pathway for ethylene synthesis. The described DNA sequences can also be used to block synthesis of ACC synthase or ACC oxidate using co-suppression. The result in either event is that the transformed plants are incapable of synthesizing ethylene, though other aspects of their metabolism is not affected.

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PURIFIED PROTEINS, RECOMBINANT DNA SEQUENCES AND PROCESSES FOR CONTROLLING THE RIPENING OF COFFEE PLANTS

5 **FIELD OF THE INVENTION**

This application relates to purified proteins, recombinant DNA sequences, hosts transformed therewith and processes for controlling the ripening of coffee plants. More particularly, this

10 application relates to purified proteins, and recombinant DNA sequences that can be used to suppress the expression of coffee fruit-specific 1-aminocyclopropane-1-carboxylic acid (ACC) synthase and ACC oxidase genes. This application further

15 relates to coffee plants transformed with such sequences, thereby rendered incapable of synthesizing ethylene necessary for ripening. Application of exogenous ethylene to plants transformed in accordance with this invention makes

20 it possible to synchronize and control fruit ripening in coffee plants.

BACKGROUND OF THE INVENTION

Coffee is prepared from the roasted beans of the plants of the genus *Coffea*, generally from the

25 species *C. arabica*. Beans are the seeds of the coffee plant and are obtained by processing the fruit, most ideally mature fruit which commands the

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best price due to its superior quality. In the
past, high quality "gourmet" coffee was hand picked.
This is necessary because the fruits of a coffee
tree do not ripen uniformly and thus there are both
5   mature and immature fruit on the same tree. In the
past, this was not a serious problem as most coffee
is grown in areas of the world where labor is
plentiful and not expensive. However, more recently
lack of abundant and inexpensive labor has become a
10 major contributor to decreased productivity in
coffee production. To increase productivity some
regions of the world, such as the largest coffee
producing country, Brazil, have resorted to strip
harvesting where workers rapidly remove all fruit
15 from a branch whether ripe or unripe. This
increases the speed of harvesting but decreases the
yield of the highest quality beans as much of the
fruit is immature (green).

Furthermore, the lack of uniform ripening has
20 seriously limited the effectiveness of mechanical
harvesting. The force required to remove mature
fruit (cherry) from the tree is similar to the force
required to remove green fruit. Thus, mechanical
harvesters do not distinguish well between green and
25 cherry and a large amount of immature fruit is
harvested along with mature fruit. This greatly

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decreases the yield of mature fruit and limits

productivity. If coffee fruit ripening could be

controlled so that all fruit ripened at one time,

both the strip method of hand harvesting and

5 mechanical harvesting would be much more efficient

and a higher percentage of the harvested fruit would

be in the higher quality grades. This would

increase profitability of coffee production.

As is the case with many other fruit [Yang and

10 Hoffman, Ann. Rev. Plant Physiol. 35:155 (1984)],

plant-produced ethylene plays an important role in

the final stages of fruit ripening in coffee. Once

coffee fruit reach a certain stage of maturity they

can be induced to ripen by the exogenous application

15 of ethylene [Crisosto, C.H., P.C. Tausend, M.A.

Nagao, L.H. Fuchigami and T.H.H. Chen, J. Haw. Pac.

Agri. 3:13-17 (1991)]. This demonstrates the

importance of ethylene for the final stages of fruit

ripening in coffee.

20   Ethylene is synthesized in a two-step reaction

from S-adenosylmethionine (SAM). The first step is

the synthesis of 1-aminocyclopropane-1-carboxylic

acid (ACC) from SAM by ACC synthase. In most plants

this is the rate limiting step. The final step is

25 the conversion of ACC to ethylene which is catalyzed

by ACC oxidase (Yang and Hoffman, *supra*).

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Inhibition of ethylene biosynthesis by chemical
(*e.g.*, silver ions or carbon dioxide) or
biotechnological means [Oeller *et al.*, Science
254:437 (1991)] inhibits the final stages of
5   ripening. This inhibition is reversible by the
application of ethylene.

   Accordingly, a strategy for controllmg the
ripening of coffee plants is to prevent synthesis of
specific enzymes in the pathway for ethylene
10 biosynthesis. In one embodiment this invention
relates to genetic alteration of coffee plants to
eliminate synthesis of ACC synthase; in another, ACC
oxidase synthesis is suppressed. In the presently
preferred embodiments, synthesis of one or both of
15 these enzymes is suppressed by transforming coffee
plants with a DNA sequence that codes on
transcription for a messenger RNA (mRNA) that is
antisense to the mRNA that codes on expression for
the enzyme whose synthesis is to be suppressed. See
20 Oeller *et al.*, Science 254:437 (1991), who reported
controlling ripening of tomatoes using a similar
strategy.

   Recombinant DNA technology has been used to
isolate a number of ACC synthase and ACC oxidase
25 genes. However, the genes for ACC synthase and ACC

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oxidase in coffee have not been identified or sequenced to date.

SUMMARY OF INVENTION

5   Purified proteins, DNA sequences that code on expression therefore and recombinant DNA molecules, including hosts transformed therewith, for transforming coffee plants to suppress the expression of enzymes necessary for ethylene

10 synthesis. The DNA sequences and recombinant DNA molecules are characterized in that they code on expression for the enzymes ACC synthase or ACC oxidase that are elements of the pathway for ethylene biosynthesis in coffee plants.

15   Coffee plants are transformed with vectors containing ACC synthase and/or with ACC oxidase DNA sequences inserted so that the transforming sequences code on expression for the respective RNA that is antisense to the mRNA for ACC synthase

20 and/or ACC oxidase. The resulting antisense RNA binds to mRNA(s), thereby inactivating the mRNA encoding one or more enzymes in the pathway for ethylene synthesis. The described DNA sequences can also be used to block synthesis of ACC synthase or

25 ACC oxidase using co-suppression. The result in either event is that the transformed plants are

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incapable of synthesizing ethylene, though other
aspects of their metabolism is not affected.

Ripening in the transformed plants can be
regulated by exogenous ethylene. By application of
5   ethylene to the entire plant, the entire plant will
ripen at once, making mechanical harvesting of
coffee more productive.

SUMMARY OF THE DRAWINGS

FIGURE 1 is the complete sequence of the cDNA
10 encoding coffee fruit expressed ACC synthase.

FIGURE 2 is the amino acid sequence of the
coffee fruit ACC synthase deduced from the cDNA
sequence shown in FIGURE 1.

FIGURE 3 is the sequence of the cDNA encoding
15 coffee fruit expressed ACC oxidase.

FIGURE 4 is the amino acid sequence of the
coffee fruit ACC oxidase deduced from the cDNA
sequence shown in FIGURE 3.

DETAILED DESCRIPTION OF THE INVENTION

20   In order that the invention herein described
may be more fully understood, the following detailed
description is set forth. In the description the
following terms are employed:

Nucleotide -- A monomeric unit of DNA or RNA
25 consisting of a sugar moiety (pentose), a phosphate,
and a nitrogenous heterocyclic base. The base is

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linked to the sugar moiety via the glycosidic carbon

(1' carbon of the pentose) and that combination of

base and sugar is called a nucleoside. The base

characterizes the nucleotide. The four DNA bases

5   are adenine ("A"), guanine ("G"), cytosine ("C"),

and thymine ("T"). The four RNA bases are A, G, C,

and uracil ("U").

   DNA Sequence -- A linear array of nucleotides

connected one to the other by phosphodiester bonds

10 between the 3' and 5' carbons of adjacent pentoses.

   Codon -- A DNA sequence of three nucleotides (a

triplet) which encodes through mRNA an amino acid, a

translation start signal or a translation

termination signal. For example, the nucleotide

15 triplets TTA, TTG, CTT, CTC, CTA and CTG encode for

the amino acid leucine ("Leu"), TAG, TAA and TGA are

translation stop signals and ATG is a translation

start signal, which also encodes the amino acid

methionine ("MET").

20   Polypeptide -- A linear array of amino acids

connected one to the other by peptide bonds between

the amino and carboxy groups of adjacent amino

acids.

   Genome -- The entire DNA of a cell or a virus.

25 It includes inter alia the structural gene coding

for the polypeptides of the substance, as well as

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promoter, transcription and translation initiation
and termination sites.

Gene -- A DNA sequence which encodes through
its template or messenger RNA ("mRNA") a sequence of
5   amino acids characteristic of a specific
polypeptide.

Transcription -- The process of producing mRNA
from a gene or DNA sequence.

Translation -- The process of producing a
10   polypeptide from mRNA.

Expression -- The process undergone by a gene
or DNA sequence to produce a polypeptide. It is a
combination of transcription and translation.

Plasmid -- A nonchromosomal double-stranded DNA
15   sequence comprising an intact "replicon" such that
the plasmid is replicated in a host cell. When the
plasmid is placed within a unicellular organism, the
characteristics of that organism may be changed or
transformed as a result of the DNA of the plasmid.
20   For example, a plasmid carrying the gene for
tetracycline resistance (TETR) transforms a cell
previously sensitive to tetracycline into one which
is resistant to it. A cell transformed by a plasmid
is called a "transformant."

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Phage or Bacteriophage -- Bacterial virus many
of which consist of DNA sequences encapsidated in a
protein envelope or coat ("capsid").

Cloning Vehicle -- A plasmid, phage DNA, cosmid

5   or other DNA sequence which is able to replicate in
a host cell, characterized by one or a small number
of endonuclease recognition sites at which such DNA
sequences may be cut in a determinable fashion
without attendant loss of an essential biological

10 function of the DNA, e.g., replication, production
of coat proteins or loss of promoter or binding
sites and which contain a marker suitable for use in
the identification of transformed cells, e.g.,
tetracycline resistance or ampicillin resistance. A

15 cloning vehicle is often called a vector.

Cloning -- The process of obtaining a
population of organisms or DNA sequences derived
from one such organism or sequence by asexual
reproduction.

20   Recombinant DNA Molecule or Hybrid DNA - A
molecule consisting of segments of DNA from
different genomes which have been joined end-to-end
outside of living cells and able to be maintained in
living cells.

25   cDNA - A DNA strand complementary to an mRNA
that codes for a particular polypeptide.

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The strategy for controlling ethylene
biosynthesis in coffee plants according to the
present invention relates in the first instance to
determination of the genes that code on expression

5   for two enzymes in the ethylene pathway: ACC
synthase and ACC oxidase. Transformation of wild
type coffee plants with constructs containing either
or both genes in an orientation that is antisense to
the normal genes is expected to block synthesis of

10   the respective enzymes. Messenger RNA transcribed
under direction from the transforming sequence will
bind to mRMA transcribed under direction from the
normal sequence, thereby inactivating the normal
message and precluding enzyme synthesis.

15   To isolate the DNA sequences that code on
expression for ACC synthase and ACC oxidase in
coffee, we screened a cDNA library produced from
coffee plant tissue with synthetic DNA probes
containing nucleotide sequences expected to occur.

20   These expected sequences were based on studies of
nucleotide sequences that occur in genes that encode
the respective enzymes, other climacteric plants and
other plants.

The cDNA corresponding to the gene encoding ACC

25   synthase or ACC oxidase is used to transform
embryonic coffee plants. The plasmid pBI-121 is

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used as a transforming vector. The sequences
corresponding to DNA that codes on expression for
ACC synthase or ACC oxidase is inserted into the
plasmid in an inverted orientation adjacent to a

5   cauliflower mosaic virus 35S promoter. RNA
transcribed therefrom will be complementary to mRNA
that encodes the amino acid sequence of the
respective enzyme. Complete constructs are
amplified in bacterial hosts. The hosts are

10 disrupted and the amplified vector is attached to
colloidal gold particles. The gold particles with
adherent vectors are inserted into coffee plant
tissue by propelling the particles at high speed at
the cells as described in U.S. patent 5,107,065.

15 Young plants successfully transformed are identified
by antibiotic resistance. The transformed plants do
not produce ACC synthase or ACC oxidase, depending
on the gene used to transform the plants. Ripening
of the transformed plants is initiated by

20 application of exogenous ethylene.

EXAMPLE 1

Isolation of Coffee Fruit-Specific ACC Synthase cDNA

In order to isolate ACC synthase gene sequences
involved in the ripening of coffee, a cDNA library

25 was prepared from a mixture of coffee fruit pericarp
and mesocarp tissue at different stages of ripeness.

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This library was screened using a PCR product synthesized from first-strand cDNA made from the same mRNA used to construct the library and degenerate oligonucleotide primers corresponding to

5 consensus sequences derived from ACC synthase genes from other organisms. This example principally involved the isolation of mRNA, the construction of a cDNA library, and the subsequent steps involved in cloning the appropriate cDNA.

10 **a)   Isolation of mRNA**

Total RNA was isolated from 66 g of pericarp and mesocarp tissue from several different developmental stages of coffee fruit (*C. arabica* L. cv Guatemalan) using the method of Levi et al.,

15 [Hort Science 27(12):1316-1318 (1992)]. Frozen coffee fruit pericarp and mesocarp tissue was powdered by grinding for about 2 minutes in a domestic coffee mill (Salton Model GC-5; Salton Maxam Housewares Group, Mt. Prospect, IL) with a

20 small piece of dry ice. The powdered fruit tissue was added to 200 μL of 200 mM tris[hydroxymethyl]aminomethane hydrochloride (tris-HCl) (pH 8.5), 1.5% sodium dodecyl sulfate (SDS), 300 mM LiCl, 10 mM disodium

25 ethylenediaminetetraacetic acid (Na$_2$EDTA), 1.5% sodium deoxycholate (w:v), 1.5% Nonidet P-40 (Sigma

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Chemical Co.) (v:v), 0.5 mM thiourea, 1 mM

aurintricarboxylic acid, 10 mM dithiothreitol (DTT),

75 mM B-mercaptoethanol, 2% polyvinylpyrrolidone

(PVP) and 2% polyvinylpoly-pyrrolidone (PVPP) and

5   homogenized using a Polytron tissue homogenizer

(Tekmar, Cincinnati, OH). After 2 minutes of

homogenization, 200 μL of chloroform was added and

homogenization continued for a further 3 minutes.

The homogenate was transferred to 250 μL centrifuge

10   bottles (Nalgene) and centrifuged for 15 minutes at

2,500 x g. The upper aqueous phase was removed and

mixed with 12 μL of 5 M NaCl, equally divided into

two centrifuge bottles, and 150 μL of ethanol was

added to each bottle. The mixture was stored at -

15   20°C overnight. The RNA was collected by

centrifugation at 4,000 x g for 15 minutes at 4°C.

The RNA was dissolved in 50 μL TE1 (50 mM tris-HCL

[pH 8.0], 10 mM Na_2EDTA) and clarified by

centrifugation at 12,000 x g for 10 minutes at 4°C.

20   The supernatant was transferred to a new centrifuge

bottle and 3 μL of 5 M NaCl and 30 μL of isopropanol

were added. The contents were mixed and stored at -

20°C overnight. The RNA was collected by

centrifugation at 14,000 x g for 10 minutes. The

25   RNA was washed with 20 μL of 70% ice-cold ethanol

and collected by centrifugation as before. After

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drying under vacuum for 10 minutes, the RNA was

resuspended in 50 μL of TE1 buffer and 10 μL of 12 M

LiCl was added. The solution was incubated at 4°C

for 48 hours and the RNA was collected by

5   centrifugation at 14,000 x g for 10 minutes and

resuspended in 30 μL TE1 buffer. After the addition

of 15 μL of 5 M potassium acetate, the RNA was

incubated overnight at 0°C, recovered by

centrifugation at 14,000 x g for 10 minutes and

10   suspended in 50 μL TE1 buffer. Three μL of 5 M NaCl

and 110 μL of 95% ethanol were added and the RNA was

incubated at -20°C overnight. The RNA was recovered

by centrifugation at 14,000 x g for 10 minutes,

washed with 20 μL of 70% ice-cold ethanol, recovered

15   by centrifugation as above, dried under vacuum for

10 minutes and resuspended in 600 μL of TE1 buffer.

The RNA was transferred into a microcentrifuge tube

and centrifuged at 14,000 rpm for 30 minutes at 4°C

after which 300 μL was removed to each of two new

20   microcentrifuge tubes. The original centrifuged

tube was rinsed with an additional 300 μL of TE1

buffer. Eighteen μL of 5 M NaCl and 636 μL of 100%

ethanol were added to each of the three tubes.

After mixing by inverting, the tubes were stored

25   overnight at -20°C. The RNA was collected by

centrifugation at 14,000 rpm for 30 minutes and

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washed with 1 μL of 70% ice-cold ethanol. After

centrifugation and drying as above, the RNA was

resuspended in 400 μL sterile H₂O. A total of 1.04

mg total RNA was obtained.

5   Messenger RNA (polyA+ RNA) was isolated using

the PolyATtract® mRNA Isolation System IV (Promega

Corporation, Madison, WI). A total of two

isolations were done as follows. For each

isolation, 0.48 mg total RNA was dissolved in 800 μL

10 of RNase-free water. After heating at 65°C for 10

minutes, 3 μL of 50 pmole/mL biotinylated oligo(dT)

and 20.7 μL of 20 X SSC (1 X SSC contains 150 mM

NaCl and 15 mM sodium citrate) were added and the

mixture was allowed to slowly cool to room

15 temperature over a period of approximately 30

minutes. An aliquot of streptavidin paramagnetic

particles (provided in the PolyATtrack® mRNA

Isolation System IV) was washed 3 times in 0.5 X SSC

and resuspended in 0.1 mL of 0.5 X SSC. The RNA

20 solution containing the biotinylated oligo(dT) was

added to the washed streptavidin paramagnetic

particles. After a 10 minute incubation at room

temperature, the paramagnetic particles containing

the trapped mRNA were captured to the side of the

25 tube using a magnet.

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The supernatant was removed and the particles

were washed four times with 0.3 mL of 0.1 X SSC.

The mRNA was removed from the biotinylated oligo(dT)

particles by suspending in 200 μL RNase-free water.

5   An additional elution was carried out by adding 150

μL of water sequentially to each of the two tubes.

The elution fractions (550 μL) were pooled and

centrifuged at 14,000 rpm in a microcentrifuge for

30 minutes at 4°C. The supernatant was divided into

10   two microcentrifuge tubes and, after the addition of

1/10th volume of 3 M NaCl and 600 μL of ethanol, the

mRNA was recovered by incubating the tubes at -20°C

overnight, followed by centrifugation as above. The

mRNA was washed once with 1 mL of ice-cold 70%

15   ethanol, dried and resuspended in 20 μL sterile H_2O.

One μL was added to 1 mL of water and a spectrum was

obtained from 230 nm through 330 nm in a Shimadzu UV

160U spectrophotometer. Approximately 6 μg of mRNA

was recovered from 1.04 mg of total RNA.

20   **b)   Construction of a cDNA Library**

First and second strand cDNA was synthesized

using the ZAP-cDNA synthesis kit (Stratagene, La

Jolla, CA). Six micrograms of mRNA in 20 μL of

water were incubated at 65°C for 5 minutes. Two

25   microliters of 100 mM methyl mercury were added and

incubation was continued at room temperature for 10

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minutes. Four microliters of 700 mM

β-mercaptoethanol were added and the incubation was

continued for an additional 5 minutes. To the

denatured mRNA, 5 µL of 10 X first strand buffer

5   (provided in the kit), 5 µL of 100 mM DTT, 3 µL

nucleotide mixture (10 mM each dATP, dGTP, dTTP and

5-methyl-dCTP), 2 µL of 1.4 µg/µL linker-primer:

5'-GAGAGAGAGAGAGAGAGAGAACTAGTCTCGAGTTTTTTTTTTTTTTTTTTT-3'

(SEQ. ID NO. 1)

10

1 µL RNase block and 5 µL of water were added. The

reaction was incubated at room temperature for 10

minutes to anneal the primer to the mRNA and then 3

15   µL of 20 U/µL M-MuLV reverse transcriptase were

added. Five microliters of this reaction mixture

were removed to a tube containing 0.5 µL (0.625

pmoles) of 800 Ci/mmole[α^{32}P]dATP. Both reactions

were incubated at 37°C for 1 hour. The

20   radioactively labeled reaction was frozen at -20° C

for later gel analysis. To the 45 µL main reaction,

40 ML of second strand buffer, 15 µL of 100 mM DTT,

6 µL of nucleotide mixture (10 mM dATP, dGTP, dTTP

and 26 mM dCTP), 268.3 µL water and 2 µL (2.5

25   pmoles) of 800 Ci/mmol [α^{32}P]dATP were added. After

mixing, 4.5 µL of 1 U/µL RNase H and 19.2 µL of 5.2

U/µL E. coli DNA polymerase I were added and the

reaction was incubated at 16° C for 2.5 hours. The

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reaction was extracted with 400 μL of phenol:chloroform (1:1). The phases were separated by centrifugation in a microcentrifuge for 5 min and the aqueous phase removed and re-extracted with

5   chloroform. The aqueous phase was recovered by centrifugation as before.

The double-stranded cDNA was precipitated by the addition of 33.3 μL of 3M sodium acetate (pH 5.2) and 867 μL of 100% ethanol and incubation

10   overnight at -20°C. The cDNA was recovered by centrifugation at 14,000 X g in a microcentrifuge at 4°C for 60 minutes. The cDNA was washed with 1 mL of 80% ethanol, recovered by centrifugation at room temperature in a microcentrifuge at 14,000 X g,

15   dried under vacuum and dissolved in 45 μL of water. Three microliters of the resuspended double-stranded cDNA was removed and stored at -20°C for later analysis by gel electrophoresis.

To the remaining 42 ML of the double-stranded

20   cDNA, 5 μL of 10 X Klenow buffer (buffer #3; supplied by Stratagene), 2.5 μL of 2.5 mM nucleotides (dCTP, dGTP, dATP and dTTP), and 0.5 μL of 5 U/μL $E.$ $coli$ DNA polymerase I Klenow fragment were added. After 30 minutes at 37°C, 50 μL of

25   water were added and the reaction was extracted with an equal volume of phenol:chloroform (1:1) and then

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chloroform as described above. After the addition

of 7 μL of 3M sodium acetate (pH 5.2) and 226 μL of

100% ethanol, the blunt-ended double-stranded cDNA

was incubated on ice for 30 minutes and recovered by

5   centrifuging at 14,000 rpm at 4°C for 60 minutes in

a microcentrifuge. The cDNA was washed with 300 μL

of 70% ethanol, centrifuged and dried as before.

Seven microliters of 0.4 μg/μL EcoRI linkers were

added to the dried cDNA. The structure of the EcoRI

10   linkers are:

5'-AATTCGGCACGAG-3' (SEQ. ID NO. 2)

3'-GCCGTGCTC-5'

After vortexing to resuspend the cDNA, 1 μL of 10 X

ligation buffer, 1 μL 10 mM ATP and 1 μL of 4 Weiss

15   U/μL T4 DNA ligase were added and the reaction was

incubated over night at 8°C. The ligase was

inactivated by heating at 70°C for 30 minutes. The

5' ends of the EcoRI linkers, that are now attached

to the cDNA, were phosphorylated using

20   polynucleotide kinase. One microliter of 10 X

buffer #3 of the ZAP-cDNA synthesis kit (Stratagene,

La Jolla, CA), 2 μL of 10 mM ATP, 6 μL of water and

1 μL of 10 U/μL T4 polynucleotide kinase were added

to the ligation reaction. After 30 minutes at 37°C

25   the kinase reaction was stopped by heating the

reaction at 70°C for 30 minutes. XhoI "sticky ends"

-20-

were generated at the end of the cDNA corresponding

to the 3' end of the mRNA by digestion of the *XhoI*

site in the linker-primer. Twenty-eight µL of *XhoI*

buffer and 3 µL of 40 U/µL *XhoI* were added to the

5   cDNA and the reaction was incubated at 37°C for 1.5

hours.

The cDNA, with *EcoRI* sticky ends at the 5' end

and *XhoI* sticky ends at the 3' end (relative to the

original mRNA), was size fractionated by passage

10 through a Sephacryl S-400 spin column prepared as

follows. Five µL of 10 X STE [100 mM Tris (pH 7.0),

5 mM EDTA and 100 mM NaCl] were added to the cDNA

and the cDNA was applied to the top of a 1 mL

syringe containing Sephacryl S-400 (Pharmacia

15 Biotech, Piscataway, NJ). A 500 µL microcentrifuge

tube was placed on the bottom of the syringe and the

column was placed in a centrifuge tube and

centrifuged at about 400 X *g* for 2 minutes. Sixty

µL of 1 X STE were added to the top of the syringe,

20 a new microcentrifuge tube was placed on the bottom

of the column and the column was again centrifuged

as before. This process was repeated until six

fractions had been collected. About 10% of each

fraction was electrophoresed on a 1% agarose gel to

25 determine the size distribution of the cDNA in each

fraction. The remainder of each fraction was

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extracted with an equal volume of phenol:chloroform
and then chloroform as described above and
precipitated by the addition of 2 volumes of 100%
ethanol. After overnight incubation at -20°C the

5   cDNA was recovered by centrifugation in a
microcentrifuge at 14,000 rpm for 60 minutes at 4°C.
Each cDNA fraction was washed with 200 NL of 80%
ethanol and dried as described above. cDNA fraction
1 was resuspended in 3 μL of sterile water, and cDNA

10   fraction 2 was resuspended in 10.5 μL of sterile
water. One-half μL of each of the two fractions was
used to determine the quantity of DNA using the
ethidium bromide plate detection method. Fractions
1 and 2, containing the largest cDNA molecules, were

15   combined. The 12.5 mL combined fractions contained
approximately 100 ng of cDNA. This fraction was
reduced to 2.5 μL in a Speed-Vac and stored on ice.
cDNA fraction 3 was resuspended in 10.5 μL of
sterile water, and saved at -20°C for later use.

20   One-hundred ng of cDNA from fraction 1 and 2
were ligated into 1 μg of Uni-ZAP™ (Stratagene, La
Jolla, CA), a lambda ZAP vector that had been
digested with EcoRI and XhoI. Fraction 1 and 2 cDNA
(2.5 μL) were added to 0.5 μL of 10 X ligation

25   buffer, 0.5 μL 10 mM ATP, 1 μL of 1 μg/μL Uni-Zap XR
vector and 0.5 μL of 4 Weiss U/μL T4 DNA ligase.

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The reaction was incubated at 8°C for about 44

hours. A 1 μL aliquot of the ligation reaction was

added to one aliquot of the 'Freeze-Thaw' extract

from the Gigapack II Gold bacteriophage λ packaging

5   kit (Stratagene, La Jolla, CA). Fifteen microliters

of Sonic extract were added and the contents were

gently mixed. The packaging was carried out at room

temperature. After 2 hours, 500 μL of SM buffer and

20 μL of chloroform were added to each packaging

10   reaction and the debris was removed by a short

centrifugation in a microcentrifuge. The packaged

phages were moved to a new microcentrifuge tube.

Ten μL of chloroform were added and the packages

phages were stored at 4°C until used. A titer of

15   this primary library indicated the presence of 0.7 X

10^6 recombinant plaques.

c)   **Amplification of primary library.**

Six-hundred μL of *E. coli* XL1-Blue MRF'

(Stratagene, La Jolla, CA), grown to a density of

20   0.5 at O.D.$_{600}$, and 32.5 μL of primary library stock

were added to each of 16 tubes. After incubation at

37°C for 15 min, 6.0 mL of 48°C top agar (5 g/L

NaCl, 2 g/L MgSO$_4$.7H$_2$O, 5 g/L yeast extract, 10 g/L

NZ amine [pH 7.5], and 0.7% agarose) were added to

25   each tube and the contents were plated on 150 X 15

mm NZY plates (5 g/L NaCl, 2 g/L MgSO$_4$.7H$_2$O, 5 g/L

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yeast extract, 10 g/L NZ amine [pH 7.5], and 15 g/L

Difco agar). The plates were incubated overnight at

37°C and then overlayed with 10 mL of SM buffer and

incubated for a further 8 hours at 4°C with gentle

5   shaking. The SM buffer was collected with a sterile

pipette and stored in a sterile 250 mL centrifuge

bottle. Each plate was rinsed with an additional 10

mL of SM buffer which were collected and added to

the previous SM buffer. Chloroform, to a final

10   concentration of 5%, was added and the phage

solution was incubated at room temperature for 15

minutes and then centrifuged at 2,000 X g for 10

minutes to remove cell debris. The supernatant was

recovered to a sterile polypropylene bottle and

15   chloroform was added to a final concentration of

0.3%. The amplified library was stored at 4°C.

**d)   Plating of amplified library for screening for
        specific genes.**

20   The amplified library was titered as described

above. Approximately 50,000 recombinant plaques

were added to 600 μL of *E. coli* XL1-Blue MRF′ that

were grown as described above. After 15 min at

37°C, 6.5 mL of 48°C top agar were added and the

25   cells were plated on 150 X 15 mm NZY plates. Four

plates containing a total of 200,000 recombinant

plaques were prepared and incubated at 37°C

overnight. The plates were then chilled for 4 hours

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at 4°C, then used for preparing plaque lifts as
described below.

   e)   Identification and Construction of
5   **Oligonucleotides Homologous to Coffee ACC**
       Synthase Genes

       In previous studies, described in United States

patent application serial number 08/485,107 the

10   specification of which is incorporated herein by

reference, we identified base sequences common to

ACC synthase occurring in a variety of plants,

referred to herein as consensus sequences. Based on

these studies, we developed a set of three (3) fully

15   degenerate primers for PCR amplification of regions

of coffee first strand cDNA corresponding to

consensus sequences. The sequence of the primers

used is:

ACS167:   5'-GCCAAGCTTCCRTGRTARTCYTGRAA-3'

20   (SEQ. ID NO. 3)

ACS289:   5'-TTYCARGAYTAYCAYGGHYT-3'

                                         (SEQ. ID NO. 4)

ACS885:   5'-CCHGGDARNCCYAWRTCTTT-3'

                                         (SEQ. ID NO. 5)

25   **f)   Reverse Transcriptase reaction to obtain first-**
       strand coffee cDNA.

       The reverse transcriptase reaction to obtain

first-strand cDNA was performed in a final volume of

30   20 μL using the GeneAmp RNA PCR Core Kit (Perkin

-25-

Elmer, Foster City, CA). First, 0.9 μg of coffee

fruit mRNA in 3 μL water was mixed with 1 μL of 50

μM random hexamer and 6 μL of sterile water in a

microcentrifuge tube and incubated at 65°C for 5

5   minutes. The mixture was left at room temperature

for 2 minutes and the liquid was recovered to the

bottom of the tube by a brief centrifugation. To

this mixture 2 μL PCR buffer II (from the above

mentioned kit), 4 μL 25 mM $MgCl_2$, 2 μL 10 mM dNTP's,

10 1 μL RNAsin (20 u/μL), and 1 μL reverse

transcriptase (50 u/μL) were added. The reaction

was incubated at 42°C for 1 hour after which the

reverse transcriptase was heat inactivated in a 95°C

water bath for 5 minutes.

15 **g)   Polymerase chain reaction to amplify coffee
        ACC-synthase gene.**

A polymerase chain reaction (PCR) (Saiki et

al., 1988) was performed using the GeneAmp Kit

20 described above in a 50 μL reaction containing 10 μL

first-strand cDNA mix, 4 μL PCR buffer II, 1 μL 25

mM $MgCl_2$, 2.5 μL of 20 μM AC5167 primer (SEQ. ID NO.

3), 2.5 μL 20 μM AC5885 primer (SEQ. ID. NO. 5),

29.5 μL sterile H_2O, and 0.5 μL Tag DNA polymerase (5

25 u/μL). PCR conditions were 35 cycles of 94°C for 1

minute, 44°C for 1 minute, and 72°C for 2 minutes.

The product of the PCR reaction was analyzed by

agarose gel electrophoresis using 1.5% SeaPlaque

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agarose (FMC BioProducts, Rockland, ME) and *Hae* III-
digested φX174 DNA (Promega Corporation, Madison,
WI) as size markers. A single PCR product of
approximately 650 bp was obtained.

5   h)   **Amplification of PCR product with different
         primers.**

The 650 bp fragment obtained above was excised
from the gel and placed in a 1.5 mL microcentrifuge

10 tube. After the addition of 200 μL of sterile
water, the 650 bp fragment was heated to 90°C for 5
minutes, cooled to room temperature and centrifuged
at 14,000 rpm for 5 minutes in a microcentrifuge.
The supernatant containing the amplified DNA was

15 removed and placed in a new sterile 1.5 mL
microcentrifuge tube. A 25 μL PCR reaction was
carried out using 0.4 μL of the previously amplified
DNA as template, 2.5 μL 10 X PCR buffer (10 mM Tris-
HCl pH 9.0, 0.1% triton X-100), 2 μL 25 mM MgCl$_2$, 5

20 μL of 1 mM dNTPs, 1 μL of 20 μM ACS289 primer (SEQ.
ID. NO. 5), 1 μL of 20 μM ACS885 primer (table 2),
12.8 μL H$_2$O, and 0.3 μL Tag DNA polymerase (5 u/μL)
(Promega Corporation, Madison, WI). The PCR was
performed using 35 cycles of 94°C for 1 minute, 45°C

25 for 1 minute, and 72°C for 2 minutes. Five μL of
this reaction was electrophoresed in a 1.5% agarose
gel as described above. A single product of
approximately 603 bp was observed. Eighty μL of

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sterile water, 10 µL of 3 M sodium acetate (pH 5.2),

and 220 µL of 100% ethanol was added to the

remainder of the reaction. After incubation at -

20°C overnight, the DNA was recovered by

5 centrifugation at 4°C for 30 minutes at 14,000 rpm.

The DNA was washed with 400 µL of ice-cold 75%

ethanol and resuspended in 25 µL of sterile water.

The DNA concentration was determined to be 10 ng/µL

using the ethidium bromide plate assay.

10 **i)   Labeling Coffee Fruit-Specific ACC Synthase DNA**

A random primed probe was produced using the

PCR-generated ACC synthase DNA and the Prime-a-Gene

Kit (Promega Corporation, Madison, WI). Two and

one-half µL of the DNA (25 ng) was added to 27.5 µL

15 of sterile water and the DNA was denatured by

boiling for 5 min. Ten µL of 5 X labeling buffer, 2

µL of unlabeled dNTP's [20 µM each; dCTP, dGTP,

dTTP], 2 µL 1 mg/mL acetylated BSA, 1 µL 5u/µL *E.*

coli DNA polymerase I Klenow fragment and 5 µL (50

20 µCi) of [α-^{32}P]dATP (3,000 Ci/mmole) (Dupont-NEN)

were added to give a final volume of 50 µL. After 1

hr at room temperature, the reaction was terminated

by the addition of 2 µL of 0.5 M Na$_2$EDTA and boiling

for 2 min.

25 **j)   Screening of amplified library with the ACC**
     synthase-specific probe.

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Plaque lifts of the four 150x15 mm NZY plates

containing 50,000 recombinant clones each were

prepared. Four 132 mm Magna nylon transfer

membranes (Micron Separations, Incorporated,

5   Westborough, MA) were wetted by placing them on

chromatography paper saturated with 5 X SSC buffer

for approximately 10 sec. The membranes were placed

on the plates containing the recombinant plaques for

5 min, removed and incubated, phage containing side

10 up, for 2 min on chromatography paper saturated with

0.5 M NaOH and 1.5 M NaCl. The membranes were then

neutralized by transferring onto chromatography

paper saturated with 0.5 M tris-HCl (pH 8.0) and 1.5

M NaCl, for 5 min. After a brief 20 sec treatment

15 on chromatography sheets saturated with 2 X SCC

containing 0.2 M tris-hcl (pH 7.5), the filters were

blotted dry. After 1 hour of air drying, DNA was

cross-linked to the membranes by treatment with

12,000 μJoules of a 260 nm UV light in a UV

20 Stratalinker 1800 (Stratagene, La Jolla, CA).

The four membranes were prehybridized at 65°C

for 2 hours in 100 mL 6 X SSPE (52.2 g/L NaCl, 8.3

g/L $NaH_2PO_4.H_2O$, 2.2 g/L Na_2EDTA, [pH 7.4]), 5 X

Denhardt's solution (1 g/L Ficoll, 1 g/L

25 polyvinylpyrrolidone, 1 g/L BSA [pentax fraction

V]), 0.5% SDS and 100 μg/mL denatured herring sperm

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DNA in a Hybaid Mark II hybridization oven (National

Labnet Company, Woodbridge, NJ) using HB-OV-BL

bottles.

Hybridization was carried out at 65°C for 12

5   hours in 10 mL of 6 X SSPE containing 0.5% SDS, 100

μg/mL denatured herring sperm DNA, and 52 μL of the

random primed probe described above. At the end of

the hybridization period the hybridization solution

was removed and the membranes were briefly washed

10   with 100 mL of 2 X SSC containing 0.5% SDS at 65°C.

They were then washed for an additional 30 min with

the same amount of fresh buffer again at 65°C. The

membranes were washed twice more for 30 min at 65°C

with 100 mL of 0.2 X SSC containing 0.5% SDS,

15   wrapped in a cellophane envelope and exposed to pre-

flashed Fuji RX_{GCU} X-ray film at -70°C for 24 hours.

Ten positive clones were obtained. The region of

the original plates corresponding to the identified

plaques were removed and placed in 1 mL of SM buffer

20   containing 20 μL chloroform. Of these ten, 5 were

re-plated at lower densities and rescreened as above

to obtain individual plaques.

k)   **Characterization of Coffee-Fruit ACC synthase
cDNA clones.**
25
The size of the putative coffee ACC synthase

cDNA clones was determined by polymerase chain

reaction using primers homologous to a portion of

-30-

the T3 and T7 promoters present in the cloning
vector and flanking the cDNA insertion site. The
sequence of the primers are:

T3: 5'-TAATACGACTCACTATAGGG-3' (SEQ. ID NO. 6)

5   T7: 5'-AATTAACCCTCACTAAAGGG-3' (SEQ. ID NO. 7)
The conditions for PCR were as described above
except that the temperature cycle was 95°C for 1
min., 50°C for 1 min. and 72°C for 2 min. Analysis
was by agarose gel electrophoresis as before.

10   The three largest clones were recovered as
phagemids by *in vivo* excision. Two hundred μL of
phage stock from a single plaque was mixed with 200
μL of *E. coli* XL1-Blue MRF' grown to a density at
O.D.$_{600}$ of 1.0. One μL of ExAssist (Stratagene, La

15   Jolla, CA) helper phage (>1 X 10^6 pfu/μL) was added
and the tubes were incubated at 37°C for 15 min.
Three mL of sterile LB broth were added and they
were incubated for 3 hours at 37°C with shaking.
After heating at 70°C for 20 min and centrifugation

20   at 1,000 X *g* for 15 min, 1 mL of the supernatant,
containing the excised pBluescript phagemid packaged
as filamentous phage particles, was transferred to a
sterile 1.5 mL microcentrifuge tube and stored at
4°C. Phagemids were recovered by adding 25 μL of

25   the stock solution to 200 μL of *E. coli* Solar cells
(Stratagene, La Jolla, CA) grown to a density of 1

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when measured at $O.D._{600}$. After incubation at 37°C

for 15 min, 200 μL of the cell mixture was plated on

100 X 15 mm NZY agar plates containing 50 μg/mL

ampicillin. The plates were incubated overnight at

5   37°C. Individual colonies were picked into 10 mL of

LB broth containing 50 μg/mL ampicillin and grown

overnight in a 37°C shaking incubator. The cells

were concentrated in a 1.5 mL sterile

microcentrifuge tube by repeated centrifugation and

10   the phasmid DNA was purified using the plasmid mini

kit from QIAGEN. The bacterial pellets were washed

with water and resuspended in 0.3 mL of buffer P1.

Next, 0.3 mL of alkaline lysis buffer P2 was added,

mixed gently, and incubated for less than 5 min at

15   room temperature. Following the addition of 0.3 mL

of chilled buffer P3 and mixing by inverting the

tubes 6 times, the extracts were incubated on ice

for 10 min and centrifuged at 14,000 rpm for 15 min

in a microcentrifuge. The supernatants were removed

20   and applied to QIAGEN-tip 20 columns that had been

previously equilibrated with 1 mL of QDT buffer.

The extracts were allowed to enter the resin of the

columns by gravity flow. Once the flow had stopped,

the columns were washed 4 times with 1 mL buffer QC.

25   The DNAs were eluted by washing the QIAGEN-tip 20

columns with 0.8 mL buffer QF which was collected

-32-

into 1.5 mL microcentrifuge tubes.   The DNA was

precipitated by the addition of 0.7 volumes (560 μL)

of isopropanol.   The tubes were immediately

centrifuged at 14,000 rpm for 30 min and the

5   supernatant carefully removed.   The pellets,

containing the DNA, were washed 20 with 1 mL of ice-

cold 70% ethanol, centrifuged as above, and air

dried for 5 min.   The DNA was resuspended in 50 μL

sterile H_2O.   The concentration of DNA from one

10   plasmid isolation was 0.1 μg/μL by fluormetric

analysis.

Sequencing reactions were performed by mixing 8

μL of phagmid DNA (0.8 μg) with 4 μL of either T3 or

T7 sequencing primers (0.8 pmol/μL).   Automated DNA

15   sequencing was carried out on these samples at the

University of Hawaii Biotechnology Service Center.

About 350 bp of sequence from both the 5' and the 3'

end of the cDNA was obtained.   New sequencing

primers were synthesized based on sequences near the

20   end of the previous sequences and used in the same

manner to complete the sequence of both strands of

the cDNA.   The complete sequence of the coffee

fruit-expressed ACC synthase cDNA is given in Figure

1.   The deduced amino acid sequence of the coffee

25   fruit-expressed ACC synthase is given in Figure 2.

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The sequence of the coffee ACC synthase cDNA

clone and deduced protein was compared with other

ACC synthase genes present in GenBank.   The cDNA

isolated from coffee fruit shows from 68.3% to 58.1%

5   identity to other ACC synthases present in GenBank.

And, the protein sequence deduced from this cDNA

shows from 67.9% to 50.5% identity to other ACC

synthases.   However, this cDNA is unique in that no

other sequence greater than 1500 bp showed greater

10   than 68.3% identity to it.

EXAMPLE 2

Isolation of Coffee Fruit-Specific ACC Oxidase

15   **a)   Synthesis of ACC Oxidase specific
        oligonucleotide primers.**

The isolation of total RNA, mRNA, and the

synthesis of coffee fruit-specific cDNA was as

20   described above.

Twelve ACC oxidase sequences, obtained from

GenBank, were aligned using the Pileup program of

GCG (Genetics Computer Group, Madison, WI).   A

region approximately 1000 bp from the translation

25   start codon was found to be conserved and a

degenerate oligonucleotide primer

        5'-TCATIGCKKCRAKIGGTTC-3' (SEQ. ID NO. 8)

corresponding to this region was synthesized.

Inosine (I) was placed at positions showing no

30   sequence conservation, since position could be any

-34-

of A, T, G or C.   Positions showing two-fold
ambiguity were prepared with mixed residues (T/G or
A/G). We also prepared a second primer homologous
to a region of the papaya fruit-expressed ACC

5   oxidase cDNA that had been previously cloned in our
laboratory and situated approximately 372 bp from
the translational start codon:

            5'-GACACTGTGGAGAGGCTGAC-3' (SEQ. ID NO. 9)
The two primers were used in a PCR reaction to

10 amplify a portion of the coffee fruit-expressed ACC
oxidase. The PCR contained 0.2 μL (10 ng) cDNA
fraction 3 (described in Example 1), 5 μL 10 X PCR
buffer, 3 μL 25 mM MgCl$_2$, 1 μL of each of the four 10
mM dNTPs, 1 μL of a 20 μM solution of each primer,

15 0.3 μL Taq DNA polymerase (promega Corporation,
Madison, WI) and 38.5 μL water. PCR conditions were
35 cycles of 94°C for 1 min, 50°C for 1 min, and
72°C for 1 min. A 5 min incubation at 72°C was
carried out after the last cycle. A 20 μL aliquot

20 of the product was electrophoresed in a 1.5% agarose
gel as described previously and revealed an
approximately 800 bp product. The DNA was excised
from the gel and mixed with 200 μL of sterile water
in a 1.5 mL microcentrifuge tube. After boiling for

25 5 min, 2 μL was used as a template in a 50 μL PCR
reaction as above using the same primers. Gel

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electrophoresis performed as described above using

20 μL of the PCR reaction indicated the presence of

a single 800 bp product. To the remaining 30 μL of

the PCR reaction 20 μL chloroform and 100 μL water

5   was added. The contents were mixed and centrifuged

for 2 minutes at 14,000 rpm in a microcentrifuge.

The upper aqueous phase containing the DNA was

removed to a clean microcentrifuge tube. A portion

of this DNA was radioactively labeled by random

10   primed synthesis as described above.

**b)   Screening of amplified library with random
        primed probe.**

The amplified coffee-fruit cDNA described in

15   Example 1 was used to prepare four 150 X 10 mm NZY

plates as previously described. Prehybridization,

hybridization and recovery of clones was as

previously described except that the ACC oxidase

sequence obtained by PCR was used as the probe.

20   **c)   Characterization of Coffee-Fruit ACC-oxidase
        cDNA clones.**

The size of the coffee ACC-oxidase cDNA clones

was determined by polymerase chain reaction using

25   primers homologous to the T3 and T7 promoters as

described in Example 1.

The sequence of the largest coffee ACC oxidase

cDNA clone was obtained as described in Example 1

and compared with ACC oxidase genes present in

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GenBank.   Figure 3 gives the sequence of the coffee

fruit-specific ACC oxidase.   Figure 4 gives the

deduced amino acid sequence of this protein.   The

cDNA was determined to encode ACC oxidase because it

5   is from 50.4% to 82.5% identical to other ACC

synthases nucleic acid sequenccs present in GenBank.

Also, the deduced protein sequence is from 32.5% to

86.5% identical to other ACC oxidases.

The foregoing examples are for illustrative

10   purposes only, and should not be viewed as limiting

the scope of applicants' invention, which is set

forth in the claims appended hereto.

SEQUENCE LISTING   37

(1)   GENERAL INFORMATION:

    (i)   APPLICANT: STILES, JOHN I.
                                    MOISYADI, ISTEFO
                                    NEUPANE, KABI R.

    (ii)   TITLE OF INVENTION: PURIFIED PROTEINS, RECOMBINANT
              DNA SEQUENCES AND PROCESSES FOR CONTROLLING THE
              RIPENING OF COFFEE

    (iii)   NUMBER OF SEQUENCES: 13

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              (E)   COUNTRY: USA
              (F)   ZIP: 44114

    (v)   COMPUTER READABLE FORM:
              (A)   MEDIUM TYPE: Diskette, 3.5 inch, 1.44 Mb
                     storage
              (B)   COMPUTER: IBM PC compatible
              (C)   OPERATING SYSTEM: MS-DOS v. 5.1
              (D)   SOFTWARE: WordPerfect v. 6.1

    (vi)   CURRENT APPLICATION DATA:
              (A)   APPLICATION NUMBER: 08/695,412
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              (C)   CLASSIFICATION: 435

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              (A)   APPLICATION NUMBER: US08/485,107
              (B)   FILING DATE: 07-JUN-1995

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(2)   INFORMATION FOR SEQ ID NO:1:

    (i)   SEQUENCE CHARACTERISTICS:
          (A)   LENGTH: 15 amino acid residues
          (B)   TYPE: amino acid
          (C)   STRANDEDNESS: N/A
          (D)   TOPOLOGY: linear

            (ii) MOLECULE TYPE: peptide

            (ix) FEATURE:
                  (A)   NAME/KEY: Fragment A
                  (B)   LOCATION: 17..1480

            (xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

            Ile Asn Tyr Ala Ser Gly Ala Ser Gly Ile Leu Asp Gln Xaa Gly
            1   5   10   15

(2) INFORMATION FOR SEQ ID NO:2:

            (i)   SEQUENCE CHARACTERISTICS:
                  (A) LENGTH: 14 amino acid residues
                  (B) TYPE: amino acid
                  (D) TOPOLOGY: linear

            (ii) MOLECULE TYPE: peptide

            (xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

            Ile Asn Tyr Ala Ser Gly Ala Ser Gly Ile Leu Asp Gln Thr
            1   5   10   14

(2) INFORMATION FOR SEQ ID NO:3:

            (i)   SEQUENCE CHARACTERISTICS:
                  (A)   LENGTH: 20 base pairs
                  (B)   TYPE: nucleic acid
                  (C)   STRANDEDNESS: single
                  (D)   TOPOLOGY: linear

            (ii) MOLECULE TYPE: OTHER NUCLEIC ACID
                  (A)   DESCRIPTION: PRIMER

            (v)   FRAGMENT TYPE: Internal

            (ix) FEATURE:
                  (A)   OTHER INFORMATION: N IS INOSINE

            (xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

                  ATNAAYTAYG CNAGYGGNGC   20

(2) INFORMATION FOR SEQ ID NO:4:

            (i)   SEQUENCE CHARACTERISTICS:
                  (A)   LENGTH: 20 base pairs
                  (B)   TYPE: nucleic acid
                  (C)   STRANDEDNESS: single
                  (D)   TOPOLOGY: linear

        (ii) MOLECULE TYPE: OTHER NUCLEAR ACID
             (A)   DESCRIPTION: PRIMER

        (v) FRAGMENT TYPE: INTERNAL

        (ix) FEATURE
             (A)   OTHER INFORMATION: N IS INOSINE

        (xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

             ATNAAYTAYG CNAGYGGNGC   20

    (2) INFORMATION FOR SEQ ID NO:5:

        (i) SEQUENCE CHARACTERISTICS:
             (A)   LENGTH: 20 base pairs
             (B)   TYPE: nucleic acid
             (C)   STRANDEDNESS: single
             (D)   TOPOLOGY: linear

        (ii) MOLECULE TYPE: OTHER NUCLEIC ACID
             (A)   DESCRIPTION: PRIMER

        (v) FRAGMENT TYPE: INTERNAL

        (ix) FEATURE
             (A)   OTHER INFORMATION: N IS INOSINE

        (xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

             CGNCCAGNCG NYTAYTTNAT   20

    (2) INFORMATION FOR SEQ ID NO:6:

        (i) SEQUENCE CHARACTERISTICS:
             (A)   LENGTH: 20 base pairs
             (B)   TYPE: nucleic acid
             (C)   STRANDEDNESS: single
             (D)   TOPOLOGY: linear

        (ii) MOLECULE TYPE: DNA (genomic)
             (A)   DESCRIPTION: PRIMER

        (v) FRAGMENT TYPE: INTERNAL

        (ix) FEATURE
             (A)   OTHER INFORMATION: N IS INOSINE

        (xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

             CGNCCYCTYG CYTAYTTNAT   20

(2)   INFORMATION FOR SEQ ID NO:7:

    (i)   SEQUENCE CHARACTERISTICS:
        (A)   LENGTH: 14 amino acid residues
        (B)   TYPE: amino acid
        (C)   STRANDEDNESS: single
        (D)   TOPOLOGY: linear

    (ii)   MOLECULE TYPE: peptide

        (v)   FRAGMENT TYPE: INTERNAL

    (ix)   FEATURE
        (D)   OTHER INFORMATION: Xaa is either Thr or Asp

    (xi)   SEQUENCE DESCRIPTION: SEQ ID NO:7:

Gln Tyr Val Pro Cys Tyr Phe Xaa Phe Ile Asp Asp Gln Asp
 1   5   10   14

(2)   INFORMATION FOR SEQ ID NO:8:

    (i)   SEQUENCE CHARACTERISTICS:
        (A)   LENGTH: 20 base pairs
        (B)   TYPE: nucleic acid
        (C)   STRANDEDNESS: single
        (D)   TOPOLOGY: linear

    (ii)   MOLECULE TYPE: OTHER NUCLEIC ACID
        (A)   DESCRIPTION: PRIMER

    (v)   FRAGMENT TYPE: Internal

    (ix)   FEATURE
        (A)   OTHER INFORMATION: N IS INOSINE

    (xi)   SEQUENCE DESCRIPTION: SEQ ID NO:8:

    CAWTATGTNC CNTGTTATTT   20

(2)   INFORMATION FOR SEQ ID NO:9:

    (i)   SEQUENCE CHARACTERISTICS:
        (A)   LENGTH: 20 base pairs
        (B)   TYPE: nucleic acid
        (C)   STRANDEDNESS: single
        (D)   TOPOLOGY: linear

    (ii)   MOLECULE TYPE: OTHER NUCLEIC ACID
        (A)   DESCRIPTION: PRIMER

    (v)   FRAGMENT TYPE: Internal

```
(ix) FEATURE
     (A)  OTHER INFORMATION:  N IS INOSINE

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

     AAWTAWCAHG GNACWTATTG      20
```

(2) INFORMATION FOR SEQ ID NO:10:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 488 amino acid residues
        (B) TYPE: amino acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: protein

    (ix) FEATURE:

       (A) NAME/KEY:CDS

       (B) LOCATION:178..1653

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

```
Met Glu Phe Ser Leu Lys Asn Glu Gln Gln Gln Leu Leu Ser Lys
 1           5               10              15

Met Ala Thr Asn Asp Gly His Gly Glu Asn Ser Pro Tyr Phe Asp
             20              25              30

Gly Trp Lys Ala Tyr Asp Ser Asp Pro Tyr His Pro Thr Arg Asn
             35              40              45

Pro Asn Gly Val Ile Gln Met Gly Leu Ala Glu Asn Gln Leu Cys
             50              55              60

Phe Asp Leu Ile Glu Glu Trp Val Leu Asn Asn Pro Glu Ala Ser
             65              70              75

Ile Cys Thr Ala Glu Gly Ala Asn Lys Phe Met Glu Val Ala Ile
             80              85              90

Tyr Gln Asp Tyr His Gly Leu Pro Glu Phe Arg Asn Ala Val Ala
             95              100             105

Arg Phe Met Glu Lys Val Arg Gly Asp Arg Val Lys Phe Asp Pro
             110             115             120

Asn Arg Ile Val Met Ser Gly Gly Ala Thr Gly Ala His Glu Thr
             125             130             135
```

42

```
Leu Ala Phe Cys Leu Ala Asp Pro Glu Asp Ala Phe Leu Val Pro
            140             145             150

Thr Pro Tyr Tyr Pro Gly Phe Asp Arg Asp Leu Arg Trp Arg Thr
            155             160             165

Gly Met Gln Leu Leu Pro Ile Val Cys Arg Ser Ser Asn Asp Phe
            170             175             180

Lys Val Thr Lys Glu Ser Met Glu Ala Ala Tyr Gln Lys Ala Gln
            185             190             195

Glu Ala Asn Ile Arg Val Lys Gly Phe Leu Leu Asn Asn Pro Ser
            200             205             210

Asn Pro Leu Gly Thr Val Leu Asp Arg Glu Thr Leu Ile Asp Ile
            215             220             225

Val Thr Phe Ile Asn Asp Lys Asn Ile His Leu Ile Cys Asp Glu
            230             235             240

Ile Tyr Ser Ala Thr Val Phe Ser Gln Pro Glu Phe Ile Ser Ile
            245             250             255

Ser Glu Ile Ile Glu His Asp Val Gln Cys Asn Arg Asp Leu Ile
            260             265             270

His Leu Val Tyr Ser Leu Ser Lys Asp Leu Gly Phe Pro Gly Phe
            275             280             285

Arg Val Gly Ile Leu Tyr Ser Tyr Asn Asp Ala Val Val Ser Cys
            290             295             300

Ala Arg Lys Met Ser Ser Phe Gly Leu Val Ser Thr Gln Thr Gln
            305             310             315

His Leu Ile Ala Ser Met Leu Ser Asp Glu Ala Phe Met Asp Lys
            320             325             330

Ile Ile Ser Thr Ser Ser Glu Arg Leu Ala Ala Arg His Gly Leu
            335             340             345

Phe Thr Arg Gly Leu Ala Gln Val Gly Ile Gly Thr Leu Lys Ser
            350             355             360

Ser Ala Gly Leu Tyr Phe Trp Met Asp Leu Arg Arg Leu Leu Arg
            365             370             375

Glu Ser Thr Phe Glu Ala Glu Met Glu Leu Trp Arg Ile Ile Ile
            380             385             390

His Glu Val Lys Leu Asn Val Ser Pro Gly Leu Ser Phe His Cys
            395             400             405
```

```
      Ser Glu Pro Gly Trp Phe Arg Val Cys Phe Ala Asn Met Asp Asp
                  410             415                     420

      Glu Ser Val Arg Val Ala Leu Arg Arg Ile His Lys Phe Val Leu
                  425             430                     435

      Val Gln Gly Lys Ala Thr Glu Pro Thr Thr Pro Lys Ser Arg Cys
                  440             445                     450

      Gly Ser Ser Lys Leu Gln Leu Ser Leu Ser Phe Arg Arg Leu Asp
                  455             460                     465

      Glu Arg Val Met Gly Ser His Met Met Ser Pro His Ser Pro Met
                  470             475                     480

      Ala Ser Pro Leu Val Arg Ala Thr
                  485
```

(2)   INFORMATION FOR SEQ ID NO:11:

    (i)   SEQUENCE CHARACTERISTICS:
        (A)   LENGTH: 2040 base pairs
        (B)   TYPE: nucleic acid
        (C)   STRANDEDNESS: single
        (D)   TOPOLOGY: linear

    (ii) MOLECULE TYPE: cDNA to mRNA

    (ix)Feature:

      (A)NAME/KEY:CDS

      (B)LOCATION:178..1653

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

```
GTAATCTCTT CTAAAATCAA CCATTCTCTT CATTCTTCAC TTGACAAGGC          50

CACTGCATTC TTCATTCTTT CTTGATATAT AGCCATTTTT TTCATTCTTT         100

CTTGATATAT AGCCATTTTT TTCATTCTTT CTTCATTCAT TGTCTGGAGA         150

AGTTGGTTGA GTTTTCTTGA AAATTCAAGC AAAACA ATG GAG TTC AGT         198
                                         Met Glu Phe Ser
                                          1


TTG AAA AAC GAA CAA CAA CAA CTC TTG TCG AAG ATG GCA ACC         240
Leu Lys Asn Glu Gln Gln Gln Leu Leu Ser Lys Met Ala Thr
 5               10               15
```

```
AAC GAT GGA CAT GGC GAA AAC TCG CCT TAT TTT GAT GGT TGG          282
Asn Asp Gly His Gly Glu Asn Ser Pro Tyr Phe Asp Gly Trp
     20                  25                  30


AAG GCA TAT GAT AGT GAT CCT TAC CAT CCC ACC AGA AAT CCT          324
Lys Ala Tyr Asp Ser Asp Pro Tyr His Pro Thr Arg Asn Pro
         35                  40                  45


AAT GGT GTT ATA CAG ATG GGA CTC GCA GAA AAT CAG TTA TGC          366
Asn Gly Val Ile Gln Met Gly Leu Ala Glu Asn Gln Leu Cys
             50                  55                  60


TTT GAT TTG ATC GAG GAA TGG GTT CTG AAC AAT CCA GAG GCT          408
Phe Asp Leu Ile Glu Glu Trp Val Leu Asn Asn Pro Glu Ala
                 65                  70


TCC ATT TGC ACA GCA GAA GGA GCG AAC AAA TTC ATG GAA GTT          450
Ser Ile Cys Thr Ala Glu Gly Ala Asn Lys Phe Met Glu Val
75                  80                  85


GCT ATC TAT CAA GAT TAT CAT GGC TTG CCA GAG TTC AGA AAT          492
Ala Ile Tyr Gln Asp Tyr His Gly Leu Pro Glu Phe Arg Asn
         90                  95                  100


GCT GTA GCA AGG TTC ATG GAG AAG GTG AGA GGT GAC AGA GTC          534
Ala Val Ala Arg Phe Met Glu Lys Val Arg Gly Asp Arg Val
             105                 110                 115


AAG TTC GAT CCC AAC CGC ATT GTG ATG AGT GGT GGG GCA ACC          576
Lys Phe Asp Pro Asn Arg Ile Val Met Ser Gly Gly Ala Thr
                 120                 125                 130


GGA GCT CAT GAA ACT CTG GCC TTC TGT TTA GCT GAC CCT GAA          618
Gly Ala His Glu Thr Leu Ala Phe Cys Leu Ala Asp Pro Glu
                 135                 140


GAT GCG TTT TTG GTA CCC ACA CCA TAT TAT CCA GGA TTT GAT          660
Asp Ala Phe Leu Val Pro Thr Pro Tyr Tyr Pro Gly Phe Asp
145                 150                 155


CGG GAT TTG AGG TGG CGA ACA GGG ATG CAA CTT CTT CCA ATT          702
Arg Asp Leu Arg Trp Arg Thr Gly Met Gln Leu Leu Pro Ile
         160                 165                 170
```

GTT TGT CGC AGC TCC AAT GAT TTT AAG GTC ACT AAA GAA TCC   744
Val Cys Arg Ser Ser Asn Asp Phe Lys Val Thr Lys Glu Ser
        175   180   185

ATG GAA GCT GCT TAT CAG AAA GCT CAA GAA GCC AAC ATC AGA   786
Met Glu Ala Ala Tyr Gln Lys Ala Gln Glu Ala Asn Ile Arg
            190   195   200

GTA AAG GGG TTC CTC TTA AAT AAT CCA TCA AAT CCA TTG GGA   828
Val Lys Gly Phe Leu Leu Asn Asn Pro Ser Asn Pro Leu Gly
                205   210

ACT GTT CTT GAC AGG GAA ACT TTG ATT GAT ATA GTC ACA TTC   870
Thr Val Leu Asp Arg Glu Thr Leu Ile Asp Ile Val Thr Phe
215   220   225

ATC AAT GAC AAA AAT ATC CAC TTG ATT TGT GAT GAG ATA TAT   912
Ile Asn Asp Lys Asn Ile His Leu Ile Cys Asp Glu Ile Tyr
        230   235   240

TCT GCC ACC GTC TTC AGC CAG CCC GAA TTC ATC AGC ATC TCT   954
Ser Ala Thr Val Phe Ser Gln Pro Glu Phe Ile Ser Ile Ser
        245   250   255

GAA ATA ATT GAG CAT GAT GTT CAA TGC AAC CGT GAT CTC ATA   996
Glu Ile Ile Glu His Asp Val Gln Cys Asn Arg Asp Leu Ile
                260   265   270

CAT CTT GTG TAT AGC CTG TCC AAG GAC TTG GGC TTC CCT GGA   1038
His Leu Val Tyr Ser Leu Ser Lys Asp Leu Gly Phe Pro Gly
                275   280

TTC AGA GTT GGC ATT TTG TAT TCA TAT AAT GAC GCT GTT GTC   1080
Phe Arg Val Gly Ile Leu Tyr Ser Tyr Asn Asp Ala Val Val
285   290   295

AGC TGT GCT AGA AAA ATG TCG AGT TTC GGC CTT GTT TCA ACA   1122
Ser Cys Ala Arg Lys Met Ser Ser Phe Gly Leu Val Ser Thr
        300   305   310

CAA ACT CAG CAT CTG ATT GCA TCA ATG TTA TCG GAC GAA GCA   1164
Gln Thr Gln His Leu Ile Ala Ser Met Leu Ser Asp Glu Ala
        315   320   325

```
TTT ATG GAC AAA ATC ATT TCC ACG AGC TCA GAG AGA TTA GCT      1206
Phe Met Asp Lys Ile Ile Ser Thr Ser Ser Glu Arg Leu Ala
            330             335                 340


GCA AGG CAT GGT CTT TTC ACA AGA GGA CTT GCT CAA GTA GGC      1248
Ala Arg His Gly Leu Phe Thr Arg Gly Leu Ala Gln Val Gly
                345             350


ATT GGC ACC TTA AAA AGC AGT GCG GGC CTT TAT TTC TGG ATG      1290
Ile Gly Thr Leu Lys Ser Ser Ala Gly Leu Tyr Phe Trp Met
355             360             365


GAC TTA AGG AGA CTC CTC AGG GAG TCC ACA TTT GAG GCA GAA      1332
Asp Leu Arg Arg Leu Leu Arg Glu Ser Thr Phe Glu Ala Glu
        370             375             380


ATG GAA CTT TGG AGG ATC ATA ATA CAT GAA GTC AAG CTC AAT      1374
Met Glu Leu Trp Arg Ile Ile Ile His Glu Val Lys Leu Asn
            385             390             395


GTT TCA CCA GGC TTA TCT TTC CAT TGC TCA GAA CCA GGA TGG      1416
Val Ser Pro Gly Leu Ser Phe His Cys Ser Glu Pro Gly Trp
                400             405             410


TTC AGA GTT TGC TTT GCC AAC ATG GAC GAC GAA AGT GTG AGA      1458
Phe Arg Val Cys Phe Ala Asn Met Asp Asp Glu Ser Val Arg
                415             420


GTT GCT CTC AGA AGA ATC CAC AAA TTT GTG CTT GTT CAG GGC      1500
Val Ala Leu Arg Arg Ile His Lys Phe Val Leu Val Gln Gly
425             430             435


AAG GCA ACA GAG CCA ACA ACT CCA AAG AGT CGC TGC GGA AGC      1542
Lys Ala Thr Glu Pro Thr Thr Pro Lys Ser Arg Cys Gly Ser
        440             445             450


AGC AAA CTT CAA CTC AGC TTA TCT TTC CGC AGA TTG GAC GAA      1584
Ser Lys Leu Gln Leu Ser Leu Ser Phe Arg Arg Leu Asp Glu
            455             460             465


AGG GTG ATG GGA TCG CAT ATG ATG TCC CCT CAC TCC CCG ATG      1626
Arg Val Met Gly Ser His Met Met Ser Pro His Ser Pro Met
            470             475             480
```

```
GCT TCA CCT TTG GTT CGG GCT ACA TAAATCATTT CTTGATCAGA    1670
Ala Ser Pro Leu Val Arg Ala Thr
                485


TCATATAGCA AAGATTCCTG AGTAAATACT CGAAACCCTT TCTGGATAAC    1720

TGAAAAGAGA GTTGTTGATT CTTTGCTGTA TCATACAAAC ACGTTACAGG    1770

CATTTTTTGG CCATCTGATG CGTGCAAATT GCATCAAATG CTTTTATTAT    1820

TGTCATATTC ATTTGTGTAC CTTGGTTTTC CTTGCCCTTC AGTCCTCCTT    1870

GTTTTTTGTT TCTTTGTTAT TATTTTCTTC CAGTTGATCA GTTAAACGAA    1920

GGAAGCTCAA TTGTTTCAAG CTATTAGTAA CAGATCATTT TGTAATAGCA    1970

ATAGTTTCAG GATTCTGAAA TGAAAGTTTA TCATTTTTCC ATCATTTTAA    2020

AAAAAAAAAA AAAAAAAAAA                                     2040
```

(2)   INFORMATION FOR SEQ ID NO:12:

     (i)   SEQUENCE CHARACTERISTICS:
          (A)   LENGTH: 318 amino acid residues
          (B)   TYPE: amino acid
          (C)   STRANDEDNESS: single
          (D)   TOPOLOGY: linear

     (ii)   MOLECULE TYPE: protein

     (ix)   FEATURE:

        (A) NAME/KEY:CDS

        (B) LOCATION:46..1003

     (xi)   SEQUENCE DESCRIPTION: SEQ ID NO:12

```
Met Ala Thr Phe Pro Leu Ile Asp Met Glu Lys Leu Asp Gly Glu
1               5               10              15

Glu Arg Ala Ala Thr Met Gly Val Ile Lys Asp Ala Cys Glu Ser
                20              25              30

Trp Gly Phe Phe Glu Val Leu Asn His Gly Ile Ser Asn Glu Leu
                35              40              45

Met Asp Thr Val Glu Arg Leu Thr Lys Glu His Tyr Lys Lys Cys
                50              55              60
```

```
Met Glu Leu Lys Phe Lys Glu Met Val Glu Ser Lys Glu Leu Glu
                 65              70              75

Ala Val Gln Thr Glu Ile Asn Asp Leu Asp Trp Glu Ser Thr Phe
                 80              85              90

Phe Leu Arg His Leu Pro Val Ser Asn Ile Ser Glu Val Pro Asp
                 95             100             105

Leu Asp Asp Glu Tyr Arg Lys Val Met Lys Glu Phe Ala Leu Gln
                110             115             120

Leu Glu Lys Leu Ala Glu Leu Leu Leu Asp Leu Leu Cys Glu Asn
                125             130             135

Leu Gly Leu Glu Lys Gly Tyr Leu Lys Lys Ala Phe Tyr Gly Thr
                140             145             150

Lys Gly Pro Thr Phe Gly Thr Lys Val Ser Asn Tyr Pro Pro Cys
                155             160             165

Pro Arg Pro Glu Leu Ile Lys Gly Leu Arg Ala His Thr Asp Ala
                170             175             180

Gly Gly Ile Ile Leu Leu Phe Gln Asp Asp Lys Val Ser Gly Leu
                185             190             195

Gln Leu Leu Lys Asp Gly Glu Trp Val Asp Val Pro Pro Met Arg
                200             205             210

His Ser Ile Val Ile Asn Ile Gly Asp Gln Leu Glu Val Ile Thr
                215             220             225

Asn Gly Lys Tyr Lys Ser Val Met His Arg Val Ile Ala Gln Pro
                230             235             240

Asp Gly Asn Arg Met Ser Leu Ala Ser Phe Tyr Asn Pro Gly Ser
                245             250             255

Asp Ala Val Ile Tyr Pro Ala Pro Ala Leu Val Glu Lys Glu Ala
                260             265             270

Glu Asp Lys Gln Ile Tyr Pro Lys Phe Val Phe Glu Asp Tyr Met
                275             280             285

Lys Leu Tyr Ala Gly Leu Lys Phe Gln Ala Lys Glu Pro Arg Phe
                290             295             300

Glu Ala Met Lys Ala Val Glu Ser Thr Val Asn Leu Gly Pro Ile
                305             310             315

Ala Thr Val
        318
```

(2)   INFORMATION FOR SEQ ID NO:13:

    (i)   SEQUENCE CHARACTERISTICS:
        (A)   LENGTH: 1320 base pairs
        (B)   TYPE: nucleic acid
        (C)   STRANDEDNESS: single
        (D)   TOPOLOGY: linear

    (ii) MOLECULE TYPE: cDNA to mRNA

    (ix)Feature:

      (A)NAME/KEY:CDS

      (B)LOCATION:46..1003

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

TGTAAACGAA GCATAAGCAC AAGCAAACAC AAACTAGAAA GAGAG ATG   48
                                                           Met
                                                            1

GCT ACA TTC CCC CTA ATC GAC ATG GAG AAG CTT GAC GGT GAA   90
Ala Thr Phe Pro Leu Ile Asp Met Glu Lys Leu Asp Gly Glu
            5   10   15

GAG AGG GCT GCC ACT ATG GGA GTC ATA AAA GAT GCT TGT GAA   132
Glu Arg Ala Ala Thr Met Gly Val Ile Lys Asp Ala Cys Glu
              20   25

AGC TGG GGC TTC TTT GAG GTG TTG AAT CAT GGG ATA TCT AAT   174
Ser Trp Gly Phe Phe Glu Val Leu Asn His Gly Ile Ser Asn
30   35   40

GAG CTC ATG GAC ACA GTG GAG AGG CTA ACA AAG GAG CAT TAC   216
Glu Leu Met Asp Thr Val Glu Arg Leu Thr Lys Glu His Tyr
    45   50   55

AAG AAA TGT ATG GAA CTA AAG TTC AAG GAA ATG GTG GAG AGC   258
Lys Lys Cys Met Glu Leu Lys Phe Lys Glu Met Val Glu Ser
        60   65   70

AAG GAA TTG GAA GCT GTT CAG ACT GAG ATC AAT GAT TTG GAC   300
Lys Glu Leu Glu Ala Val Gln Thr Glu Ile Asn Asp Leu Asp
        75   80   85

50

CLAIMS

1.   A substantially pure ACC synthase from *Coffea arabica* consisting essentially of the amino acid sequence: (SEQ. ID. NO. 10)

5   2.   Substantially pure nucleic acid sequence that codes on expression for the ACC synthase produced by *Coffea arabica* comprising: (SEQ. ID. NO. 11)

3.   The substantially pure nucleic acid

10   sequence that codes on expression for the ACC synthase produced by *Coffea arabica* of claim 2, wherein the nucleic acid sequence is limited to the coding regions of SEQ. ID. No. 11.

4.   A method for controlling the ripening of

15   *Coffea arabica* comprising:

a)   transforming coffee plants with a DNA sequence that is antisense to the DNA sequence SEQ. ID. NO. 11;

b)   growing plants transformed with the

20   DNA sequence of a) above; and

c)   applying exogenous ethylene to the transformed plants after coffee fruit has matured.

5.   The method for controlling the ripening of

25   *Coffea arabica* of claim 4, wherein the DNA sequence used for transforming is limited to a sequence that is antisense to the coding region of SEQ. ID. No. 11.

6.   The method for controlling fruit ripening

30   of claims 4 or 5, wherein gaseous ethylene is applied to the entire plant, to cause ripening of substantially all of the fruit simultaneously.

7.   A substantially pure ACC oxidase from *Coffea arabica* consisting essentially of the amino

35   acid sequence: (SEQ. ID. NO. 12).

8.   Substantially pure nucleic acid sequence
that codes an expression for *Coffee arabica* ACC
oxidase comprising: (SEQ. ID. No. 13).

9.   The substantially pure nucleic acid
5 sequence that codes on expression for ACC oxidase
produced by *Coffee arabica* of claim 8, wherein the
nucleic acid sequence is limited to the coding
regions of SEQ. ID. No. 13.

10.   A method for controlling ripening of
10 *Coffea arabica* comprising:

a)   transforming coffee plants with a DNA
sequence that is antisense to the DNA sequence:
(SEQ. ID. No. 13);

b)   growing plants transformed with the
15   DNA sequence of a) above; and

c)   applying exogenous ethylene to the
transformed plants after coffee fruit has
matured.

11.   The method for controlling ripening of
20 *Coffea arabica* of claim 10, wherein the DNA sequence
used for transforming is limited to a sequence that
is antisense to the coding region of SEQ. ID. NO.
13.

12.   The method for controlling fruit ripening
25 of claims 9 and 10, wherein the gaseous ethylene is
applied to the entire plant, to cause ripening of
substantially all of the fruit simultaneously.

13.   A coffee plant having suppressed
expression of ACC synthase.
30   14.   A coffee plant having suppressed
expression of ACC oxidase.

15.   A coffee plant having suppressed
expression of ACC synthase and suppressed expression
of ACC oxidase.

52

16.   A coffee plant comprising a DNA sequence
that codes on transcription for a mRNA that is
antisense to the mRNA that codes on expression for
ACC synthase.

5   17.   A coffee fruit from the coffee plant of
claim 16.

18.   A coffee bean from the coffee plant of
claim 16.

19.   A coffee plant comprising a DNA sequence
10   that codes on transcription for a mRNA that is
antisense to the mRNA that codes on expression for
ACC oxidase.

20.   A coffee fruit from the coffee plant of
claim 19.

15   21.   A coffee bean from the coffee plant of
claim 19.

22.   A coffee plant comprising (i) a first DNA
sequence that codes on transcription for a mRNA that
is antisense to the mRNA that codes on expression
20   for ACC synthase, and (ii) a second DNA sequence
that codes on transcription for a mRNA that is
antisense to the mRNA that codes on expression for
ACC oxidase.

23.   A coffee fruit from the coffee plant of
25   claim 22.

24.   A coffee bean from the coffee plant of
claim 22.

25.   A coffee plant comprising a DNA sequence
that is antisense to all or part of the DNA sequence
30   specified in SEQ ID NO:11.

26.   A coffee plant comprising a DNA sequence
that is antisense to all or part of the DNA sequence
specified in SEQ ID NO:13.

27.   A coffee plant comprising (i) a first DNA
35   sequence that is antisense to all or part of the DNA

53

sequence specified in SEQ ID NO:11, and (ii) a
second DNA sequence that is antisense to all or part
of the DNA sequence specified in SEQ ID NO:13.

28. A coffee plant produced by the process of
5   inserting into the plant genome a DNA sequence that
codes on transcription for a mRNA that is antisense
to the mRNA that codes on expression for ACC
synthase.

29. A coffee plant produced by the process of
10   inserting into the plant genome a DNA sequence that
codes on transcription for a mRNA that is antisense
to the mRNA that codes on expression for ACC
oxidase.

30. A coffee plant produced by the process of
15   inserting into the plant genome (i) a DNA sequence
that codes on transcription for a mRNA that is
antisense to the mRNA that codes on expression for
ACC synthase, and (ii) a DNA sequence that codes on
transcription for a mRNA that is antisense to the
20   mRNA that codes on expression for ACC oxidase.

31. A method for transforming a coffee plant
with a DNA sequence that codes on transcription for
a mRNA that is antisense to the mRNA that codes on
expression for ACC synthase, comprising the steps
25   of:

providing a transforming vector comprising
a DNA sequence that codes on expression for ACC
synthase, wherein the DNA sequence is inserted
into the transforming vector in an inverted
30   orientation; and

inserting the transforming vector into the
tissue of the coffee plant, wherein the
inverted DNA sequence thereafter becomes
inserted into the genome of the coffee plant.

54

32. A method for transforming a coffee plant
with a DNA sequence that codes on transcription for
a mRNA that is antisense to the mRNA that codes on
expression for ACC oxidase, comprising the steps of:

5   providing a transforming vector comprising
a DNA sequence that codes on expression for ACC
oxidase, wherein the DNA sequence is inserted
into the transforming vector in an inverted
orientation;

10   inserting the transforming vector into the
tissue of the coffee plant, wherein the
inverted DNA sequence thereafter becomes
inserted into the genome of the coffee plant.

33. A method for transforming a coffee plant

15 with (i) a first DNA sequence that codes on
transcription for a mRNA that is antisense to the
mRNA that codes on expression for ACC synthase, and
(ii) a second DNA sequence that codes on
transcription for a mRNA that is antisense to the

20 mRNA that codes on expression for ACC oxidase,
comprising the steps of:

       providing a first transforming vector
comprising a first DNA sequence that codes on
expression for ACC synthase, wherein the first

25 DNA sequence is inserted into the first
transforming vector in an inverted orientation;
       providing a second transforming vector
comprising a second DNA sequence that codes on
expression for ACC oxidase, wherein the second

30 DNA sequence is inserted into the second
transforming vector in an inverted orientation;
       inserting the first transforming vector
into the tissue of the coffee plant, wherein
the first inverted DNA sequence thereafter

55

becomes inserted into the genome of the coffee
plant; and

     inserting the second transforming vector
into the tissue of the coffee plant, wherein
5   the second inverted DNA sequence thereafter
becomes inserted into the genome of the coffee
plant.

Fig 1: DEDUCED AMINO ACID SEQUENCE OF ACC SYNTHASE FROM *COFFEA*

ARABICA (SEQ. ID. NO. 10)

```
        Met Glu Phe Ser Leu Lys Asn Glu Gln Gln Gln Leu Leu Ser Lys
         1               5                  10                  15

5

        Met Ala Thr Asn Asp Gly His Gly Glu Asn Ser Pro Tyr Phe Asp Gly
                     20                  25                  30


        Trp Lys Ala Tyr Asp Ser Asp Pro Tyr His Pro Thr Arg Asn Pro Asn

10                   35                  40                  45


        Gly Val Ile Gln Met Gly Leu Ala Glu Asn Gln Leu Cys Phe Asp Leu
                     50                  55                  60


15      Ile Glu Glu Trp Val Leu Asn Asn Pro Glu Ala Ser Ile Cys Thr Ala
                  65                  70                  75


        Glu Gly Ala Asn Lys Phe Met Glu Val Ala Ile Tyr Gln Asp Tyr His
         80                  85                  90                  95

20

        Gly Leu Pro Glu Phe Arg Asn Ala Val Ala Arg Phe Met Glu Lys Val
                     100                 105                 110


        Arg Gly Asp Arg Val Lys Phe Asp Pro Asn Arg Ile Val Met Ser Gly

25                   115                 120                 125
```

```
     Gly Ala Thr Gly Ala His Glu Thr Leu Ala Phe Cys Leu Ala Asp Pro
             130                 135                 140

     Glu Asp Ala Phe Leu Val Pro Thr Pro Tyr Tyr Pro Gly Phe Asp Arg
5            145                 150                 155

     Asp Leu Arg Trp Arg Thr Gly Met Gln Leu Leu Pro Ile Val Cys Arg
     160                 165                 170                 175

10   Ser Ser Asn Asp Phe Lys Val Thr Lys Glu Ser Met Glu Ala Ala Tyr
                     180                 185                 190

     Gln Lys Ala Gln Glu Ala Asn Ile Arg Val Lys Gly Phe Leu Leu Asn
                     195                 200                 205

15
     Asn Pro Ser Asn Pro Leu Gly Thr Val Leu Asp Arg Glu Thr Leu Ile
                 210                 215                 220

     Asp Ile Val Thr Phe Ile Asn Asp Lys Asn Ile His Leu Ile Cys Asp
20           225                 230                 235

     Glu Ile Tyr Ser Ala Thr Val Phe Ser Gln Pro Glu Phe Ile Ser Ile
             240                 245                 250                 255

25   Ser Glu Ile Ile Glu His Asp Val Gln Cys Asn Arg Asp Leu Ile His
                     260                 265                 270
```

Leu Val Tyr Ser Leu Ser Lys Asp Leu Gly Phe Pro Gly Phe Arg Val
            275   280   285

     Gly Ile Leu Tyr Ser Tyr Asn Asp Ala Val Val Ser Cys Ala Arg Lys
5   290   295   300

     Met Ser Ser Phe Gly Leu Val Ser Thr Gln Thr Gln His Leu Ile Ala
             305   310   315

     Ser Met Leu Ser Asp Glu Ala Phe Met Asp Lys Ile Ile Ser Thr Ser
10   320   325   330   335

     Ser Glu Arg Leu Ala Ala Arg His Gly Leu Phe Thr Arg Gly Leu Ala
                         340   345   350

15   Gln Val Gly Ile Gly Thr Leu Lys Ser Ser Ala Gly Leu Tyr Phe Trp
                 355   360   365

     Met Asp Leu Arg Arg Leu Leu Arg Glu Ser Thr Phe Glu Ala Glu Met
                 370   375   380

20
     Glu Leu Trp Arg Ile Ile Ile His Glu Val Lys Leu Asn Val Ser Pro
             385   390   395

     Gly Leu Ser Phe His Cys Ser Glu Pro Gly Trp Phe Arg Val Cys Phe
25   400   405   410   415

```
        Ala Asn Met Asp Asp Glu Ser Val Arg Val Ala Leu Arg Arg Ile His
                         420                 425                 430


        Lys Phe Val Leu Val Gln Gly Lys Ala Thr Glu Pro Thr Thr Pro Lys
 5                       435                 440                 445


        Ser Arg Cys Gly Ser Ser Lys Leu Gln Leu Ser Leu Ser Phe Arg Arg
                     450                 455                 460


10      Leu Asp Glu Arg Val Met Gly Ser His Met Met Ser Pro His Ser Pro
                 465                 470                 475


        Met Ala Ser Pro Leu Val Arg Ala Thr
        480                 485
```

5/12

Fig 2.: Coffee fruit-expressed ACC synthase gene sequence. (SEQ. ID. NO. 11)

```
       GTAATCTCTT CTAAAATCAA CCATTCTCTT CATTCTTCAC TTGACAAGGC CACTGCATTC        60

       TTCATTCTTT CTTGATATAT AGCCATTTTT TTCATTCTTT CTTGATATAT AGCCATTTTT       120

   5   TTCATTCTTT CTTCATTCAT TGTCTGGAGA AGTTGGTTGA GTTTTCTTGA AAATTCAAGC       180


       AAAACA ATG GAG TTC AGT TTG AAA AAC GAA CAA CAA CAA CTC TTG TCG AAG      231


       ATG GCA ACC AAC GAT GGA CAT GGC GAA AAC TCG CCT TAT TTT GAT GGT        279
  10

       TGG AAG GCA TAT GAT AGT GAT CCT TAC CAT CCC ACC AGA AAT CCT AAT        327


       GGT GTT ATA CAG ATG GGA CTC GCA GAA AAT CAG TTA TGC TTT GAT TTG        375


  15   ATC GAG GAA TGG GTT CTG AAC AAT CCA GAG GCT TCC ATT TGC ACA GCA        423


       GAA GGA GCG AAC AAA TTC ATG GAA GTT GCT ATC TAT CAA GAT TAT CAT        471


       GGC TTG CCA GAG TTC AGA AAT GCT GTA GCA AGG TTC ATG GAG AAG GTG        519
  20

       AGA GGT GAC AGA GTC AAG TTC GAT CCC AAC CGC ATT GTG ATG AGT GGT        567


       GGG GCA ACC GGA GCT CAT GAA ACT CTG GCC TTC TGT TTA GCT GAC CCT        615


  25   GAA GAT GCG TTT TTG GTA CCC ACA CCA TAT TAT CCA GGA TTT GAT CGG        663


       GAT TTG AGG TGG CGA ACA GGG ATG CAA CTT CTT CCA ATT GTT TGT CGC        711


       AGC TCC AAT GAT TTT AAG GTC ACT AAA GAA TCC ATG GAA GCT GCT TAT        759
  30

       CAG AAA GCT CAA GAA GCC AAC ATC AGA GTA AAG GGG TTC CTC TTA AAT        807
```

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          AAT CCA TCA AAT CCA TTG GGA ACT GTT CTT GAC AGG GAA ACT TTG ATT          855

          GAT ATA GTC ACA TTC ATC AAT GAC AAA AAT ATC CAC TTG ATT TGT GAT          903

    5     GAG ATA TAT TCT GCC ACC GTC TTC AGC CAG CCC GAA TTC ATC AGC ATC          951

          TCT GAA ATA ATT GAG CAT GAT GTT CAA TGC AAC CGT GAT CTC ATA CAT          999

          CTT GTG TAT AGC CTG TCC AAG GAC TTG GGC TTC CCT GGA TTC AGA GTT         1047

   10     GGC ATT TTG TAT TCA TAT AAT GAC GCT GTT GTC AGC TGT GCT AGA AAA         1095

          ATG TCG AGT TTC GGC CTT GTT TCA ACA CAA ACT CAG CAT CTG ATT GCA         1143

   15     TCA ATG TTA TCG GAC GAA GCA TTT ATG GAC AAA ATC ATT TCC ACG AGC         1191

          TCA GAG AGA TTA GCT GCA AGG CAT GGT CTT TTC ACA AGA GGA CTT GCT         1239

          CAA GTA GGC ATT GGC ACC TTA AAA AGC AGT GCG GGC CTT TAT TTC TGG         1287

   20     ATG GAC TTA AGG AGA CTC CTC AGG GAG TCC ACA TTT GAG GCA GAA ATG         1335

          GAA CTT TGG AGG ATC ATA ATA CAT GAA GTC AAG CTC AAT GTT TCA CCA         1383

   25     GGC TTA TCT TTC CAT TGC TCA GAA CCA GGA TGG TTC AGA GTT TGC TTT         1431

          GCC AAC ATG GAC GAC GAA AGT GTG AGA GTT GCT CTC AGA AGA ATC CAC         1479

          AAA TTT GTG CTT GTT CAG GGC AAG GCA ACA GAG CCA ACA ACT CCA AAG         1527

   30     AGT CGC TGC GGA AGC AGC AAA CTT CAA CTC AGC TTA TCT TTC CGC AGA         1575
```

```
     TTG GAC GAA AGG GTG ATG GGA TCG CAT ATG ATG TCC CCT CAC TCC CCG        1623


     ATG GCT TCA CCT TTG GTT CGG GCT ACA TAAATCATTT CTTGATCAGA TCATATAGCA   1680


 5   AAGATTCCTG AGTAAATACT CGAAACCCTT TCTGGATAAC TGAAAAGAGA GTTGTTGATT       1740
     CTTTGCTGTA TCATACAAAC ACGTTACAGG CATTTTTTGG CCATCTGATG CGTGCAAATT       1800
     GCATCAAATG CTTTTATTAT TGTCATATTC ATTTGTGTAC CTTGGTTTTC CTTGCCCTTC       1860
     AGTCCTCCTT GTTTTTTGTT TCTTTGTTAT TATTTTCTTC CAGTTGATCA GTTAAACGAA       1920
     GGAAGCTCAA TTGTTTCAAG CTATTAGTAA CAGATCATTT TGTAATAGCA ATAGTTTCAG       1980
10   GATTCTGAAA TGAAAGTTTA TCATTTTTCC ATCATTTTAA AAAAAAAAAA AAAAAAAAAA       2040
```

Note: The coding portion of this sequence is shown by grouping the
bases as codons.

Fig. 3: DEDUCED PROTEIN SEQUENCE OF THE COFFEE FRUIT-EXPRESSED ACC

OXIDASE cDNA (SEQ. ID. NO. 12)

```
                                                   Met Ala Thr Phe
                                                    1
5

     Pro Leu Ile Asp Met Glu Lys Leu Asp Gly Glu Glu Arg Ala Ala Thr
      5               10              15                      20

     Met Gly Val Ile Lys Asp Ala Cys Glu Ser Trp Gly Phe Phe Glu Val
10                    25              30                      35

     Leu Asn His Gly Ile Ser Asn Glu Leu Met Asp Thr Val Glu Arg Leu
                      40              45                  50

15   Thr Lys Glu His Tyr Lys Lys Cys Met Glu Leu Lys Phe Lys Glu Met
                  55                  60                  65

     Val Glu Ser Lys Glu Leu Glu Ala Val Gln Thr Glu Ile Asn Asp Leu
                  70                  75                  80
20

     Asp Trp Glu Ser Thr Phe Phe Leu Arg His Leu Pro Val Ser Asn Ile
          85              90                  95                  100

     Ser Glu Val Pro Asp Leu Asp Asp Glu Tyr Arg Lys Val Met Lys Glu
25                    105             110                     115
```

```
        Phe Ala Leu Gln Leu Glu Lys Leu Ala Glu Leu Leu Leu Asp Leu Leu
                    120                 125                 130


        Cys Glu Asn Leu Gly Leu Glu Lys Gly Tyr Leu Lys Lys Ala Phe Tyr
    5               135                 140                 145


        Gly Thr Lys Gly Pro Thr Phe Gly Thr Lys Val Ser Asn Tyr Pro Pro
                    150                 155                 160


    10  Cys Pro Arg Pro Glu Leu Ile Lys Gly Leu Arg Ala His Thr Asp Ala
        165                 170                 175                 180


        Gly Gly Ile Ile Leu Leu Phe Gln Asp Asp Lys Val Ser Gly Leu Gln
                            185                 190                 195

    15

        Leu Leu Lys Asp Gly Glu Trp Val Asp Val Pro Pro Met Arg His Ser
                    200                 205                 210


        Ile Val Ile Asn Ile Gly Asp Gln Leu Glu Val Ile Thr Asn Gly Lys
    20              215                 220                 225


        Tyr Lys Ser Val Met His Arg Val Ile Ala Gln Pro Asp Gly Asn Arg
                    230                 235                 240


    25  Met Ser Leu Ala Ser Phe Tyr Asn Pro Gly Ser Asp Ala Val Ile Tyr
        245                 250                 255                 260
```

```
     Pro Ala Pro Ala Leu Val Glu Lys Glu Ala Glu Asp Lys Gln Ile Tyr
                     265             270             275


     Pro Lys Phe Val Phe Glu Asp Tyr Met Lys Leu Tyr Ala Gly Leu Lys
5                    280             285             290


     Phe Gln Ala Lys Glu Pro Arg Phe Glu Ala Met Lys Ala Val Glu Ser
                     295             300             305


10   Thr Val Asn Leu Gly Pro Ile Ala Thr Val
                     310             315
```

Fig 4: DNA SEQUENCE OF THE COFFEE FRUIT-EXPRESSED ACC OXIDASE CDNA.

(SEQ. ID. NO. 13)

```
      TGTAAACGAA GCATAAGCAC AAGCAAACAC AAACTAGAAA GAGAG ATG GCT ACA TTC        57

  5   CCC CTA ATC GAC ATG GAG AAG CTT GAC GGT GAA GAG AGG GCT GCC ACT         105

      ATG GGA GTC ATA AAA GAT GCT TGT GAA AGC TGG GGC TTC TTT GAG GTG         153

      TTG AAT CAT GGG ATA TCT AAT GAG CTC ATG GAC ACA GTG GAG AGG CTA         201
 10
      ACA AAG GAG CAT TAC AAG AAA TGT ATG GAA CTA AAG TTC AAG GAA ATG         249

      GTG GAG AGC AAG GAA TTG GAA GCT GTT CAG ACT GAG ATC AAT GAT TTG         297

 15   GAC TGG GAA AGT ACC TTC TTC TTG CGC CAT CTT CCT GTT TCC AAC ATC         345

      TCA GAA GTC CCT GAT CTT GAT GAT GAA TAC AGA AAG GTT ATG AAG GAA         393

      TTT GCG TTG CAA CTT GAG AAA CTA GCA GAG CTC CTG TTG GAC TTG CTA         441
 20
      TGC GAG AAC CTT GGC CTA GAG AAA GGC TAT CTG AAG AAA GCC TTC TAT         489

      GGC ACC AAA GGA CCA ACC TTT GGC ACC AAA GTC AGC AAT TAC CCT CCA         537

 25   TGC CCT CGT CCA GAA CTG ATC AAG GGC CTC CGG GCA CAC ACC GAT GCC         585

      GGC GGC ATC ATC CTG CTG TTC CAG GAT GAC AAG GTC AGC GGT CTC CAG         633

      CTC CTC AAG GAT GGT GAA TGG GTG GAT GTT CCG CCT ATG CGC CAC TCC         681
 30
      ATT GTA ATC AAC ATC GGC GAC CAA CTT GAG GTA ATC ACA AAT GGA AAA         729
```

```
     TAC AAG AGT GTG ATG CAC CGG GTG ATA GCT CAA CCA GAT GGG AAC AGA          777

     ATG TCA CTA GCA TCA TTC TAC AAT CCA GGA AGT GAT GCA GTG ATC TAT          825

5    CCA GCA CCG GCA TTG GTT GAG AAA GAG GCA GAG GAC AAG CAG ATA TAT          873

     CCC AAG TTT GTG TTC GAG GAC TAC ATG AAG CTC TAT GCT GGC CTT AAG          921

     TTC CAA GCT AAA GAG CCC AGG TTT GAA GCC ATG AAG GCC GTG GAA AGC          969

10
     ACC GTA AAC TTG GGT CCA ATC GCA ACT GTT TGAGATAATA CACGCTTTGA           1019

     TCTGCTGCTG TCTTATAATG CGCGTTTGCG TAATCATATC CTAGCATAGT ATATCTGAGA       1079

15   TCTGAGTCTG TATTGTGGTG TGAGTTTGGT TTAGCCCCTT GTTAATGCTT GGATTGGACT       1139

     AGTTAAATGT GGAGCTGGTT TGTTAGATAA GATAGTCTTG CCAGGATCTT TGAGTAAATA       1199

     TGATTCTGCG GAAGTCTGCG GTGAATGATA ACGTGTAAAG CAATCCGAAA GTTACCTTTC       1259

20
     TGGGGCTTTG TCATATGCAA TGGAGAAGGA ATCTTCCAAA AAAAAAAAAA AAAAAAAAAA       1319

     A                                                                       1320
```

25   Note: The coding portion of this sequence is shown by grouping the bases as codons.

INTERNATIONAL SEARCH REPORT

A. CLASSIFICATION OF SUBJECT MATTER

IPC 6   C12N15/52   C12N15/53   C12N15/11   C12N15/82   C12N9/02
C12N9/88   C12N5/10   A01H5/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6   C12N   A01H

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 96 19103 A (DNA PLANT TECHN CORP) 27 June 1996 * see esp. pp.16-19, pp.23/24, ex.9,10 *	13-21, 28,29, 31,32
A	WO 91 01375 A (ICI PLC) 7 February 1991 see the whole document	1-33
A	WO 92 04456 A (US OF AMARICA REPRESENTED BY T) 19 March 1992 see the whole document	1-33
A	WO 96 07742 A (ASGROW SEED CO ;BOESHORE MAURY L (US); DENG ROSALINE Z (US); CARNE) 14 March 1996 see the whole document	1-33

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[X] Further documents are listed in the continuation of box C.   [X] Patent family members are listed in annex.

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"A" document defining the general state of the art which is not considered to be of particular relevance

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"O" document referring to an oral disclosure, use, exhibition or other means

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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
2 December 1997	19/12/1997

Name and mailing address of the ISA	Authorized officer
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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category *	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
A	WO 96 21027 A (ASGROW SEED CO ;BOESHORE MAURY L (US); DENG ROSALINE Z (US); CARNE) 11 July 1996 see the whole document -----	1-33

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INTERNATIONAL SEARCH REPORT

...formation on patent family members

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WO 9621027 A	11-07-96	AU 2769395 A	24-07-96
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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category '	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
A	WO 96 21027 A (ASGROW SEED CO ;BOESHORE MAURY L (US); DENG ROSALINE Z (US); CARNE) 11 July 1996 see the whole document -----	1-33

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